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Characterization of Extract of P. Notatumisolated from Virgin Forest

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Abstract- *Penicillium notatum* has been found years ago to be one of the leading novel candidates of fungi for the discovery of novel antibiotics. The fungus was isolated from eighty years old virgin forest Ikota, Ondo state. The fraction was obtained through column chromatography using mixture of solvents, after culturing and purification process of the extract. The fraction was characterized using gas chromatography mass spectrophotometer, the Gc-Ms results revealed Benzene, 1,2,4,5-trimethyl- having retention time 5.117,% of total 6.505%, p-cymene having retention time 5.170 minutes, % of total 16.439%, Trans-Decalin, 2 methyl with retention time 5.235 minutes and % of total 9.468 %, also n-Nonadecanol-1 having retention time 5.301 minutes and 13.302 %, Naphthalene with retention time 6.161 minutes, % of total 11.369 %, 5, 8,11,14-Eicosatetraenoic acid, methyl ester, having retention time of 6.381 minutes and % of total 2.544 %. Literature reports had indicated the physiological activities of these compounds and of interest p-cymene and Naphthalene derivative to possess strong pharmaceutical uses.

Keywords: *penicillium notatum, gas chromatography mass spectrophotometer, retention time, % of total.*

GJMR-B Classification : *NLMC Code: QV 252*

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I. Introduction

Fungi are rich sources of bioactive compounds. The medicinal chemists have always tried to design drug substance possessing maximum therapeutic application and minimum toxicity. Soil is traditionally the main source of fungal genetic resources for bio-prospection programs. To expand the search for pharmacologically active agent, soil sample from virgin forest of 80 years old in Ikota, Nigeria was examined.

II. Methodology

Figure 1: Showing the map of study area

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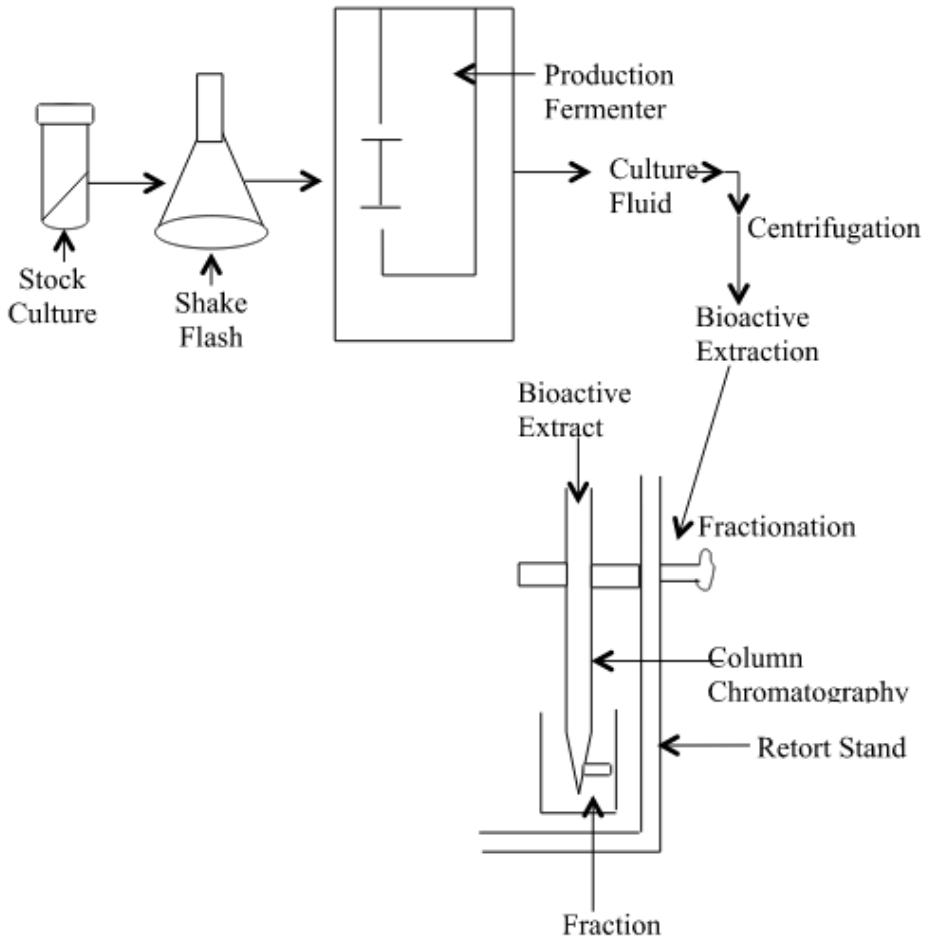

Figure 2 : Showing the flowsheet for the process

a) Soil sample collection

Soil sample collection was done in July 2013 in Ikota, Ondo State, Nigeria. Random sampling method was used in collecting soil sample directly and the collection was done using soil auger at a depth of 10cm. The collected soil was put into polythene bag and stored inside refrigerator.

b) Isolation of the fungi

One gram of soil sample was transferred to a sterile Erlenmeyer (EM) flask containing 50ml sterile water. The flask was shaken on rotary shaker for 30 minutes for the detachment of the spore chains. The flask was kept aside for 30 minutes to settle down the particulate matter. The clear supernatant was diluted with sterile water (dilutions 10^{-1} – 10^{-3}) was used on innocular. One ml of each of these dilutions was pipette out into the medium, plated into Petri dishes 6mm diameter and incubated at 28^{0}C for 2-4 weeks and potato dextrose agar was used.

c) Identification

Cultural observation: using the natural eyes and microscope at low power magnification (x40), parameters such as, colony color, color change in the medium, characteristic of the submerged hyphae whether rhizoid, spiral or regular and characteristic shape of mature fruiting bodies are strictly observed.

d) Microscopic observation

A small piece of mycelium free of medium was transferred using inoculating needle on to a glass slide containing a drop of cotton blue in loctophenol and the mycelium was spread properly with another needle. The preparation was covered with a cover slip and observed under medium power (x100) and later at high power (x400) magnifications. Details of spore colouration, shape, septation and surface marking were studied and *P. Notatum*was identified and confirmed by professor of microbiology in Microbiology laboratory.

e) Culturing the fungi

The fungi with strong antagonistic efficacy were culture in the laboratory for maximum yield of bioactive compounds using the fabricated fermenter, five ml of already cultured fungi was put into sterilized potato broth of 500ml and poured into the fermenter and allowed to excrete maximum yield for two weeks, the air pump supplied continuously sterilized air and the culture being mixed together using powered mixer.

f) Extraction and Purification

The fungi cultures were centrifuged and extraction of compounds from multiplied fungi was carried out in a separating funnel using ethyl acetate. The extract was concentrated using rotary evaporator. The fraction was eluted using mixture of 50% ethyl acetate and 50% hexane through column chromatography and the fractions was concentrated using rotary evaporator.

g) Gas Chromatography- Mass Spectrophotometer (GC-MS)

Analysis was conducted using an HP (Hewlett Packard, 5890 series И GC hyphenated with 5989 Mass Spectrometer). MS conditions were as follows: Detector mass spectrometer voltage 70eV and its source temperature was 300oC. The injector temperature was 240^{0}C and the split less mode 0.5µL injection. The HP 55% dimethyl-95% diphenylpolysiloxane non-polar column was performed with length 30 cm x 0.25 mm, coating thickness film 0.25 µm. The oven was adjusted at 100^{0}C for 1 min and initial time 1.5 min with 40^{0}C which ended by a final temperature of 300^{0}C and 4 min hold time where the total run time was 45 min. The components were identified by comparing their retention times with those of authentic samples, as well as by comparing their mass spectra with those of (NIST).

III. Results

Area Percent Report

Data Path : C:\msdchem\1\data\ADEWOLE.D\ADEWOLE, E\
Data File : AJIBOLA A1.D
Acq On : 10 Oct 2013 10:46
Operator : Ibitoye
Sample : AJIBOLA A1
Misc :
ALS Vial : 1 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 12 % of largest Peak
Start Thrs: 0.001 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 0

Method : C:\msdchem\1\data\ADEWOLE 1\ADEWOLE 3.D\FUNGI EXTRACT.M
Title :

Signal : TIC: AJIBOLA A1.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.117	3	5	10	rVB2	505342	608963	39.57%	6.505%
2	5.170	10	14	20	rVB	825111	1538826	100.00%	16.439%
3	5.235	20	25	30	rBV2	485960	886244	57.59%	9.468%
4	5.301	30	36	50	rVB7	243445	964358	62.67%	10.302%
5	5.461	50	63	67	rBV4	298830	870704	56.58%	9.302%
6	5.508	67	71	77	rBV3	305035	509795	33.13%	5.446%
7	5.633	89	92	99	rVB	236563	455632	29.61%	4.867%
8	5.710	99	105	111	rVB	358885	719703	46.77%	7.688%
9	5.971	138	149	157	rVB4	101928	355735	23.12%	3.800%
10	6.161	168	181	192	rVB2	369992	1064226	69.16%	11.369%
11	6.381	204	218	224	rVB8	63553	238098	15.47%	2.544%
12	6.826	287	293	308	rVB2	121041	317340	20.62%	3.390%
13	32.466	4596	4613	4636	rBV2	146500	605388	39.34%	6.467%
14	40.206	5900	5917	5928	rBV2	35362	225771	14.67%	2.412%

Sum of corrected areas: 9360783

FUNGI EXTRACT.M Thu Oct 10 11:52:13 2013

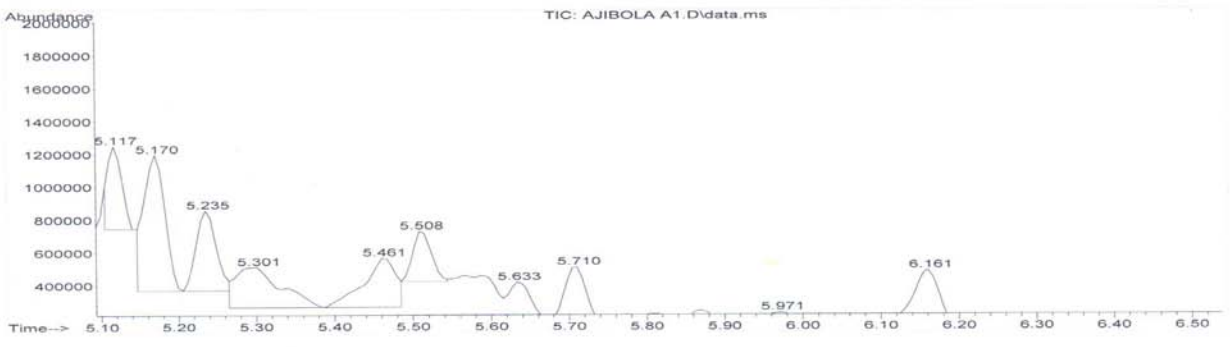

Figure 3 : Showing the chromatogram of the extract

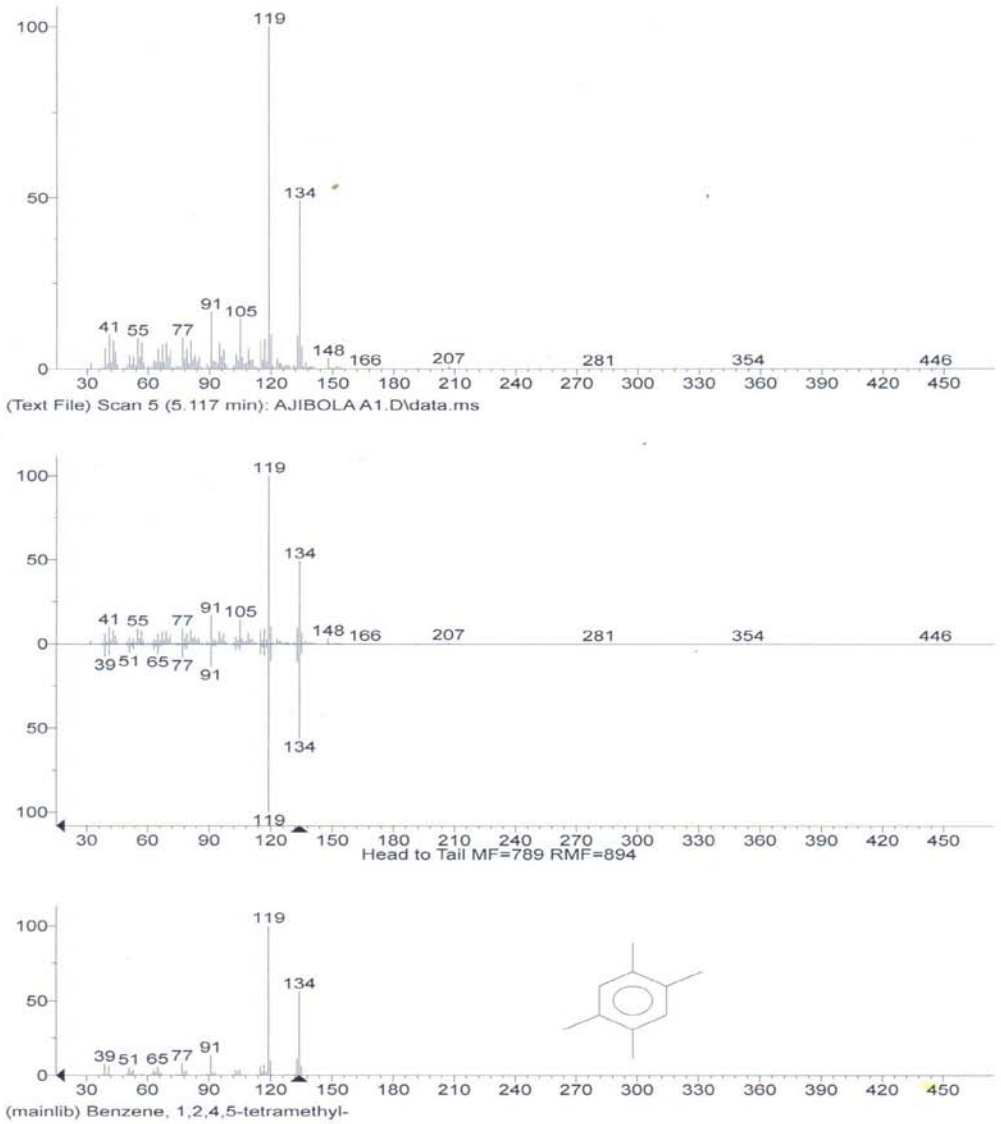

Figure 4 : Showing spectral of compound identified

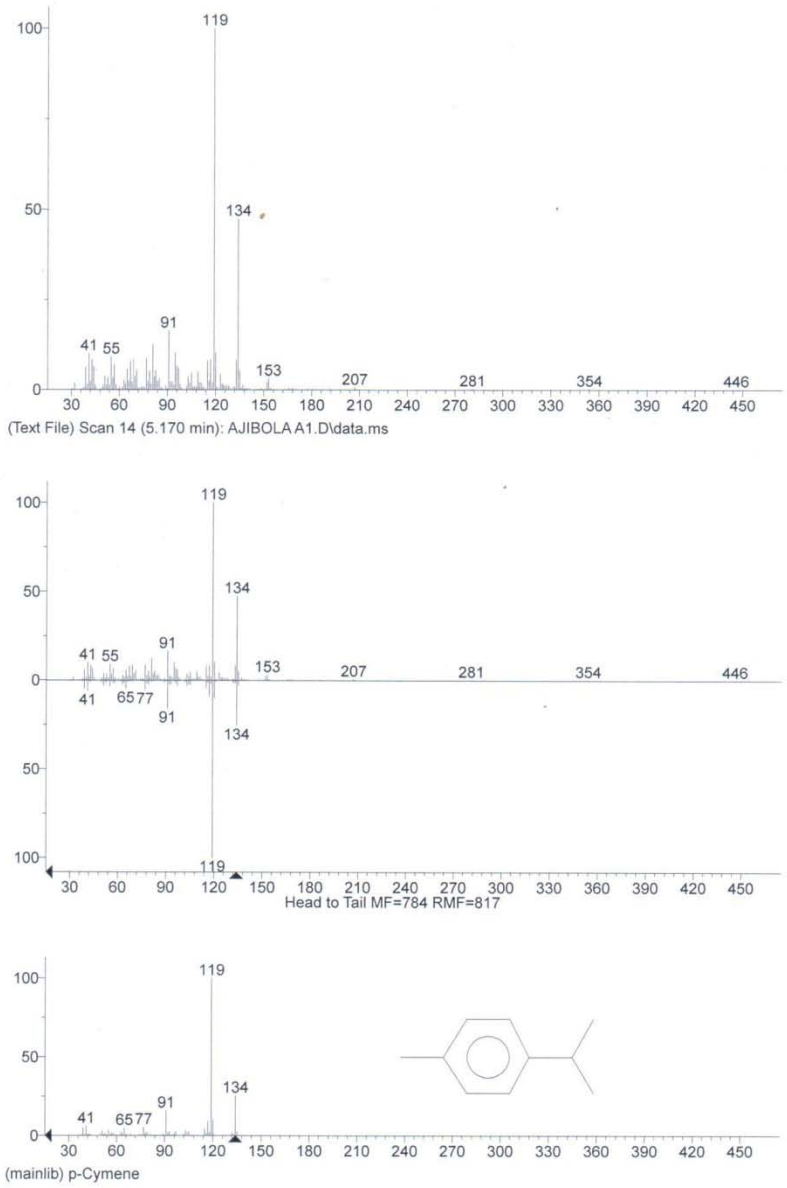

Figure 5 : Showing spectral of compound identified

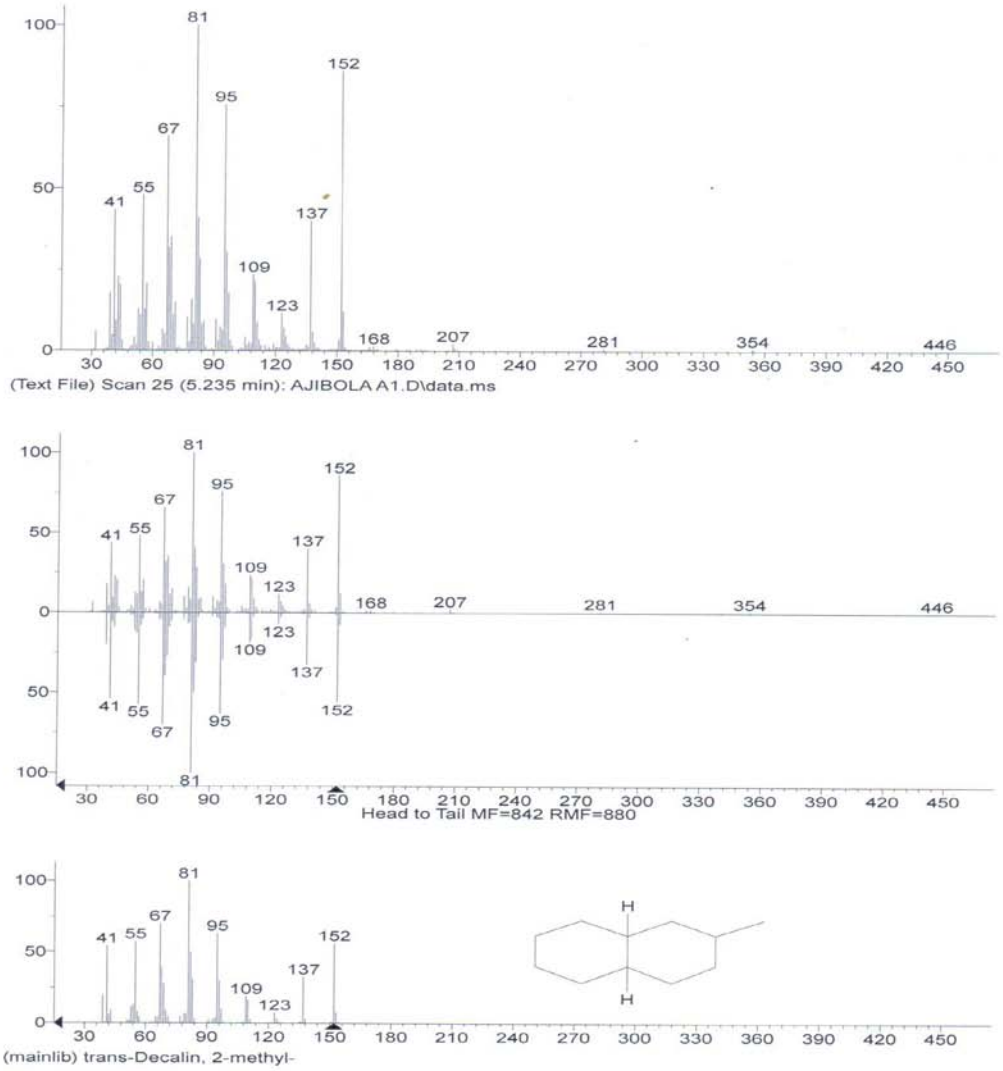

Figure 6 : Showing spectral of compound identified

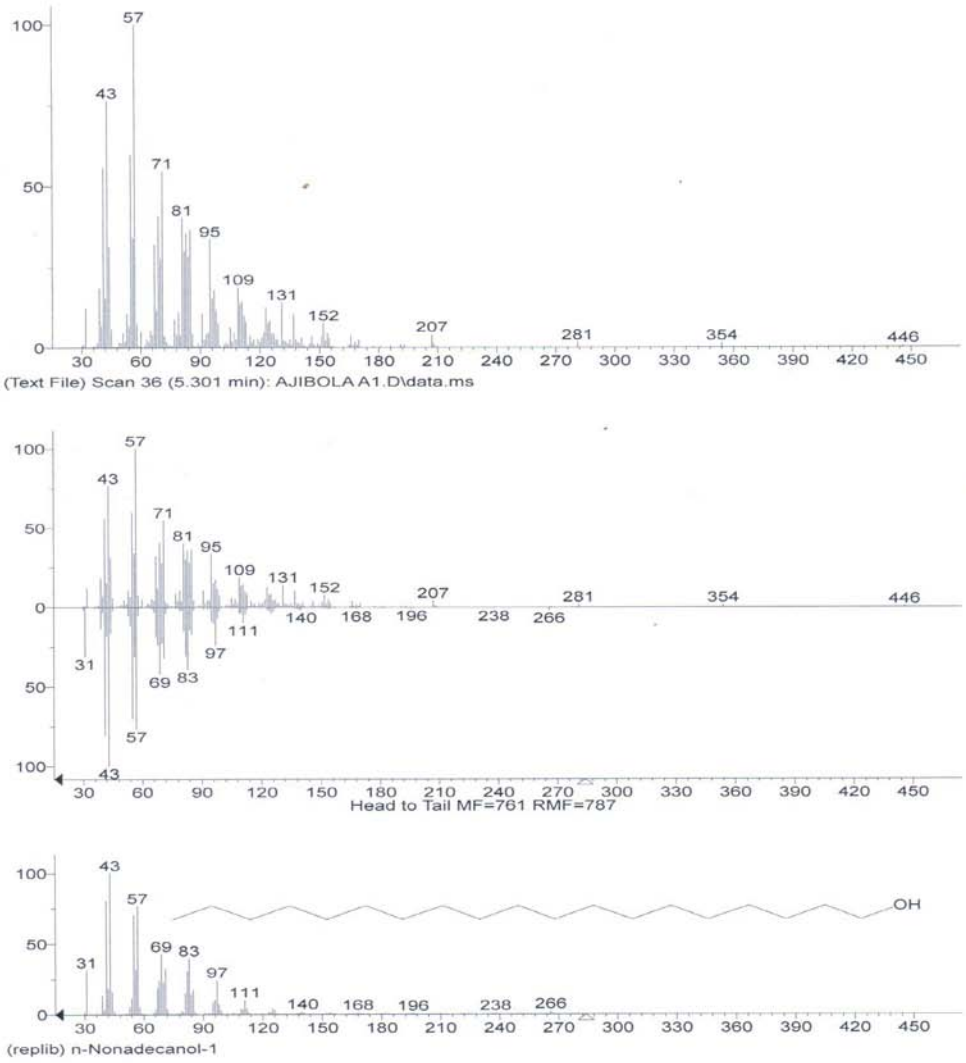

Figure 7 : Showing spectral of compound identified

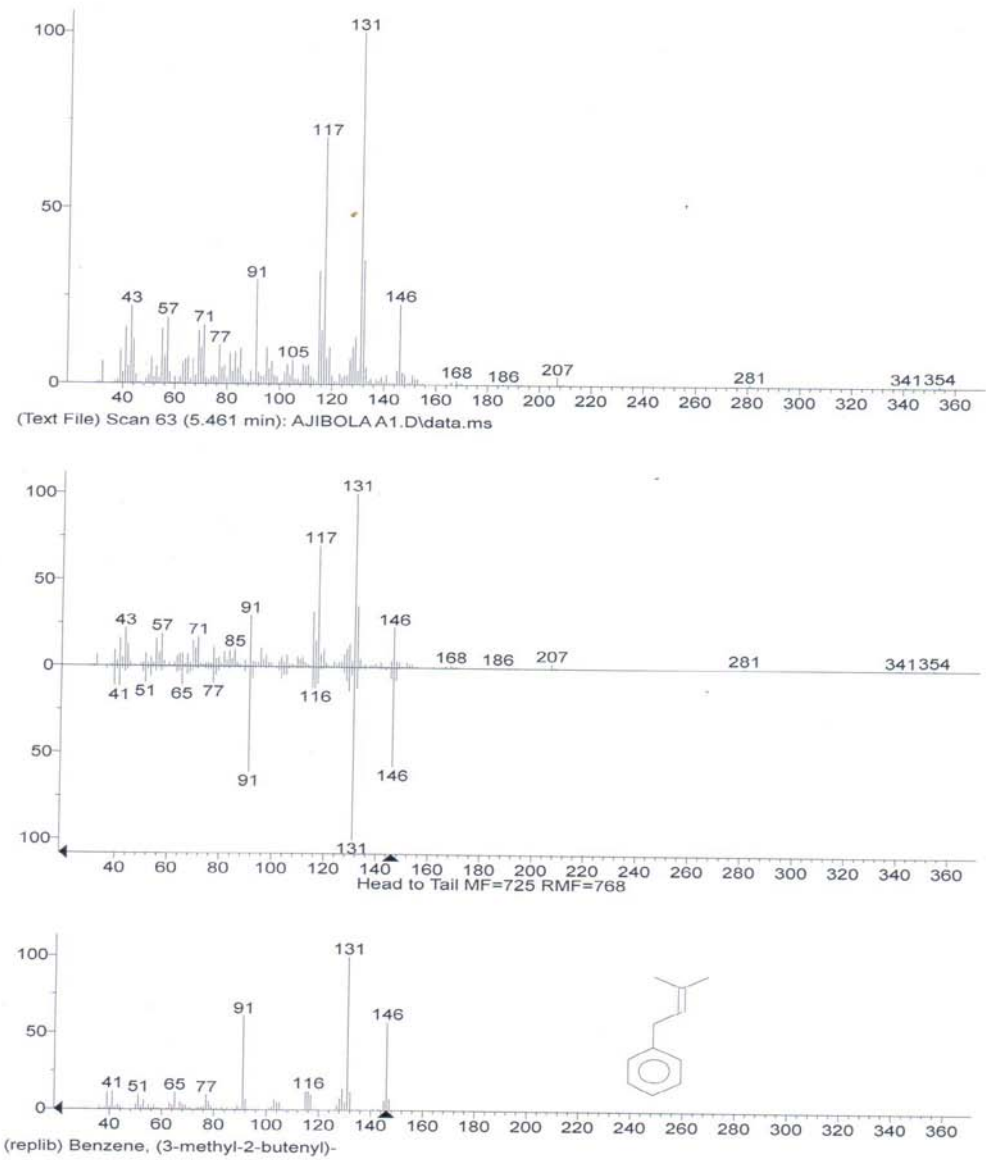

Figure 8 : Showing spectral of compound identified

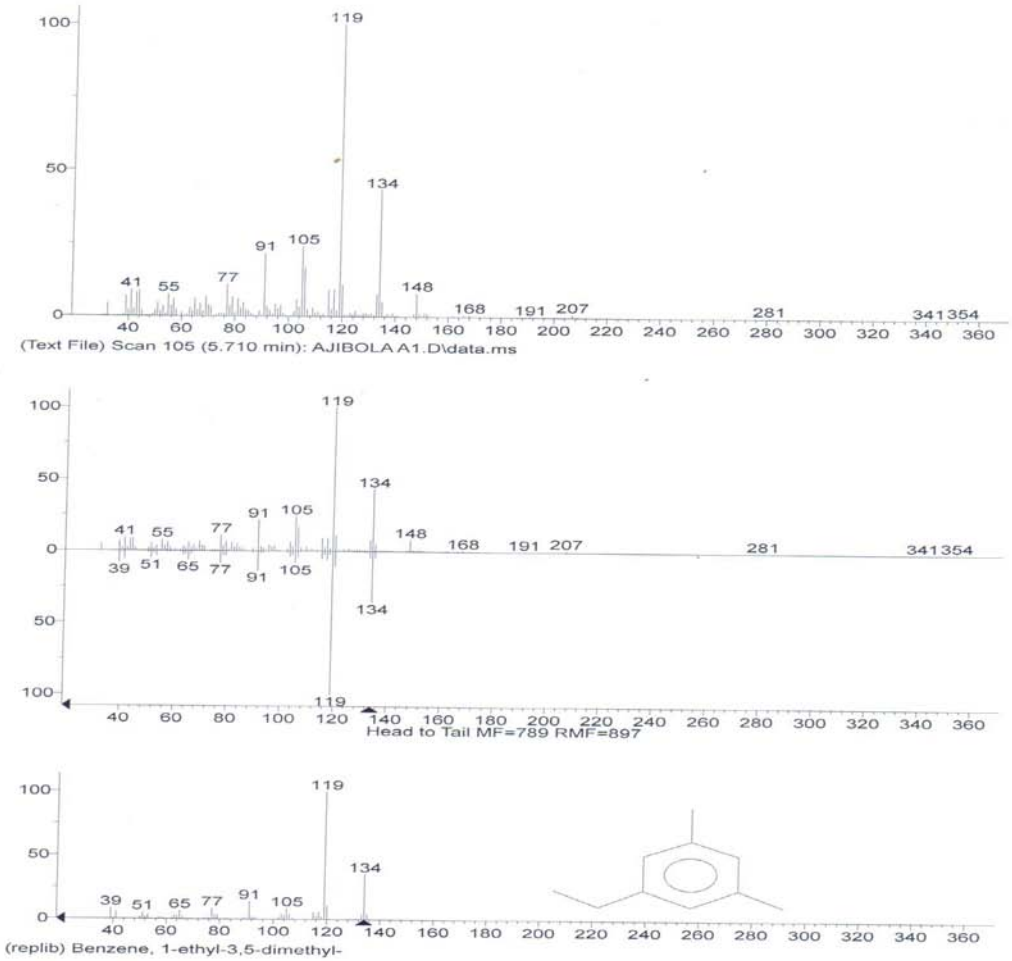

Figure 9 : Showing spectral of compound identified

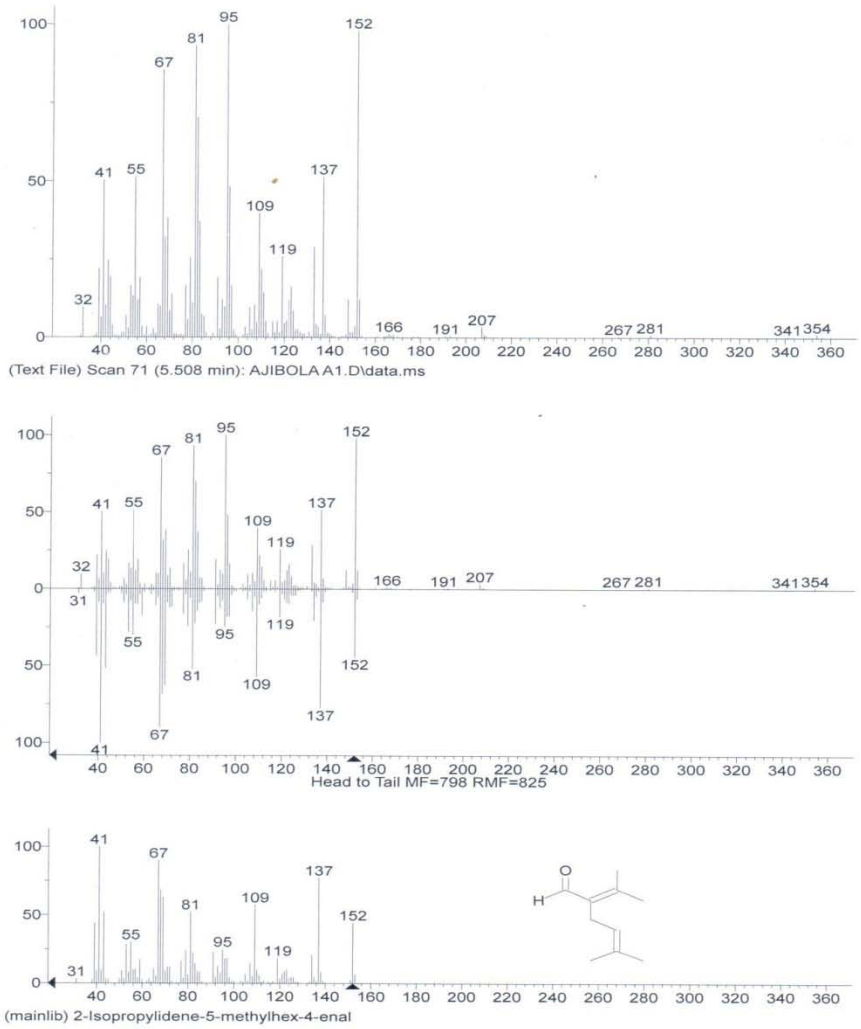

Figure 10 : Showing spectral of compound identified

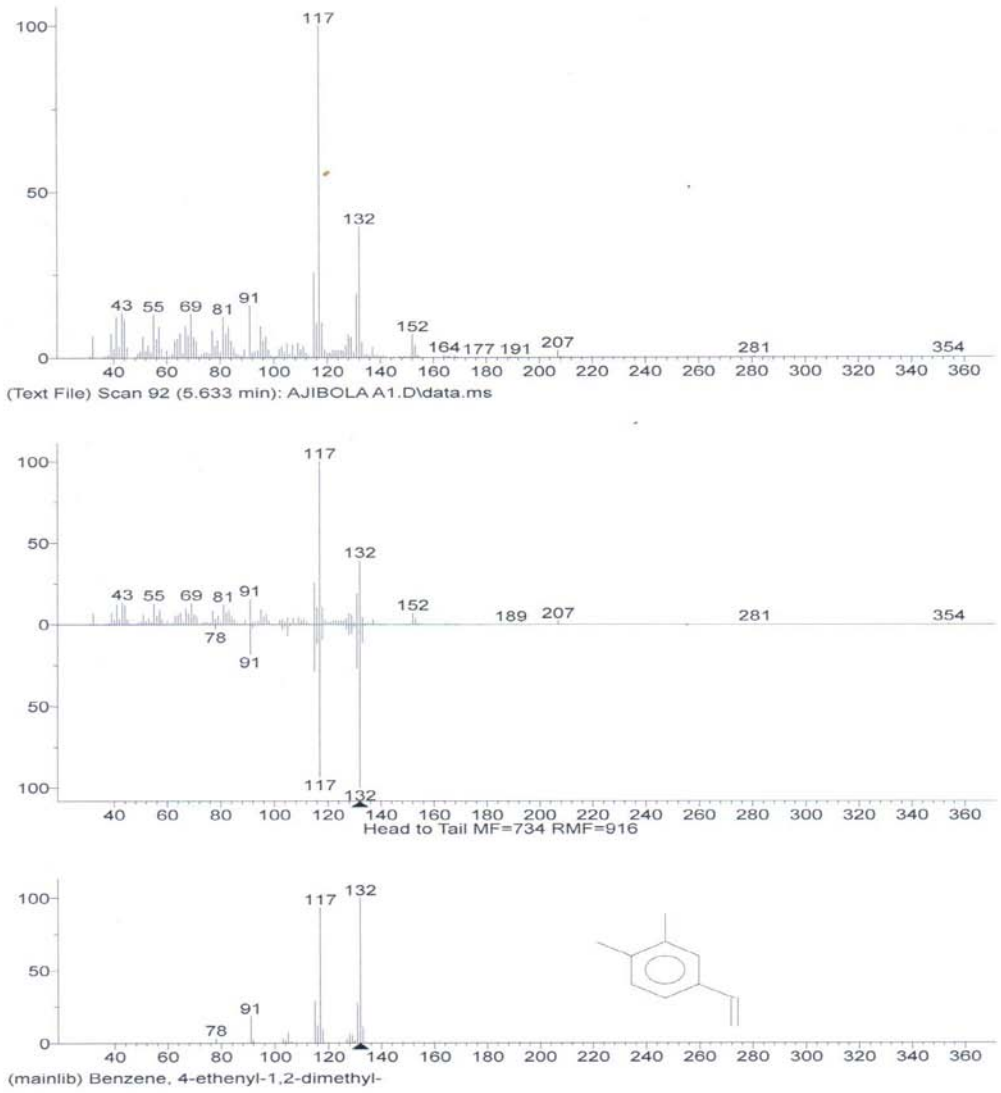

Figure 11: Showing spectral of compound identified

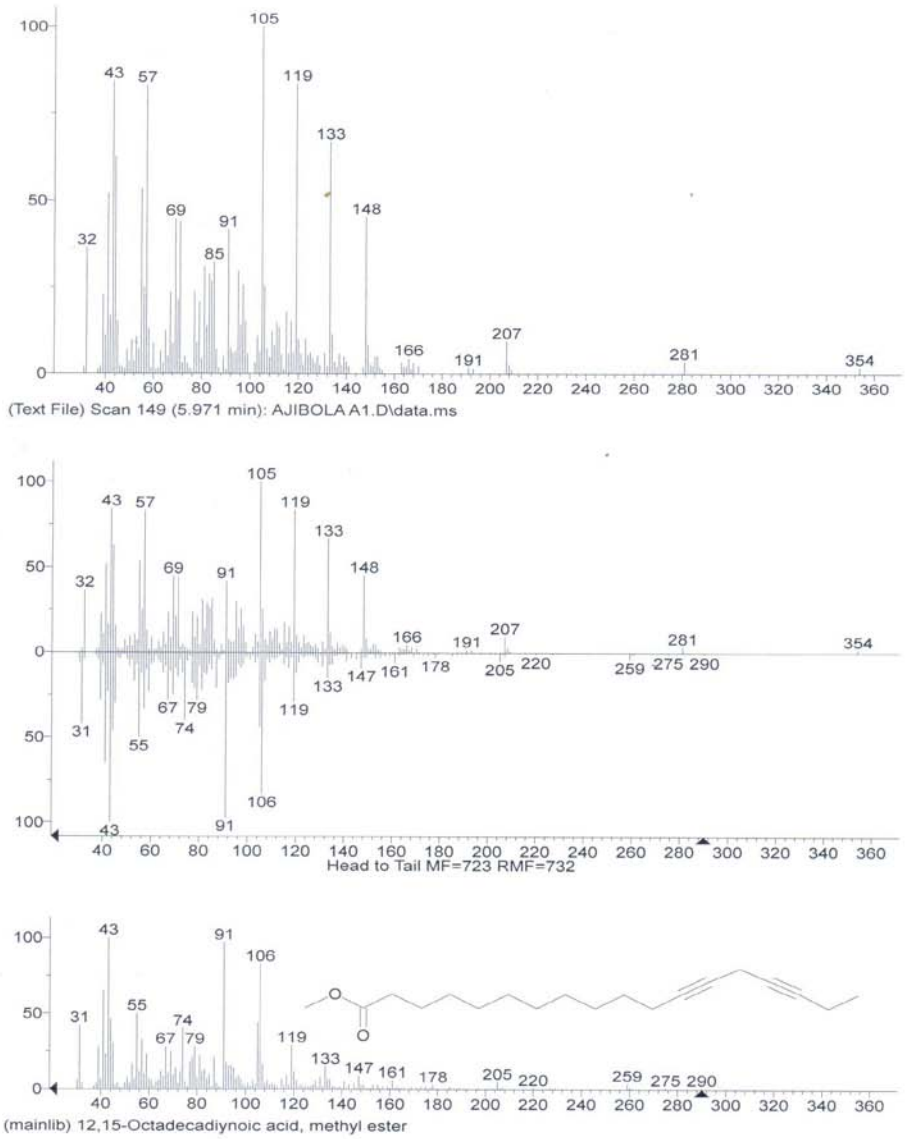

Figure 12 : Showing spectral of compound identified

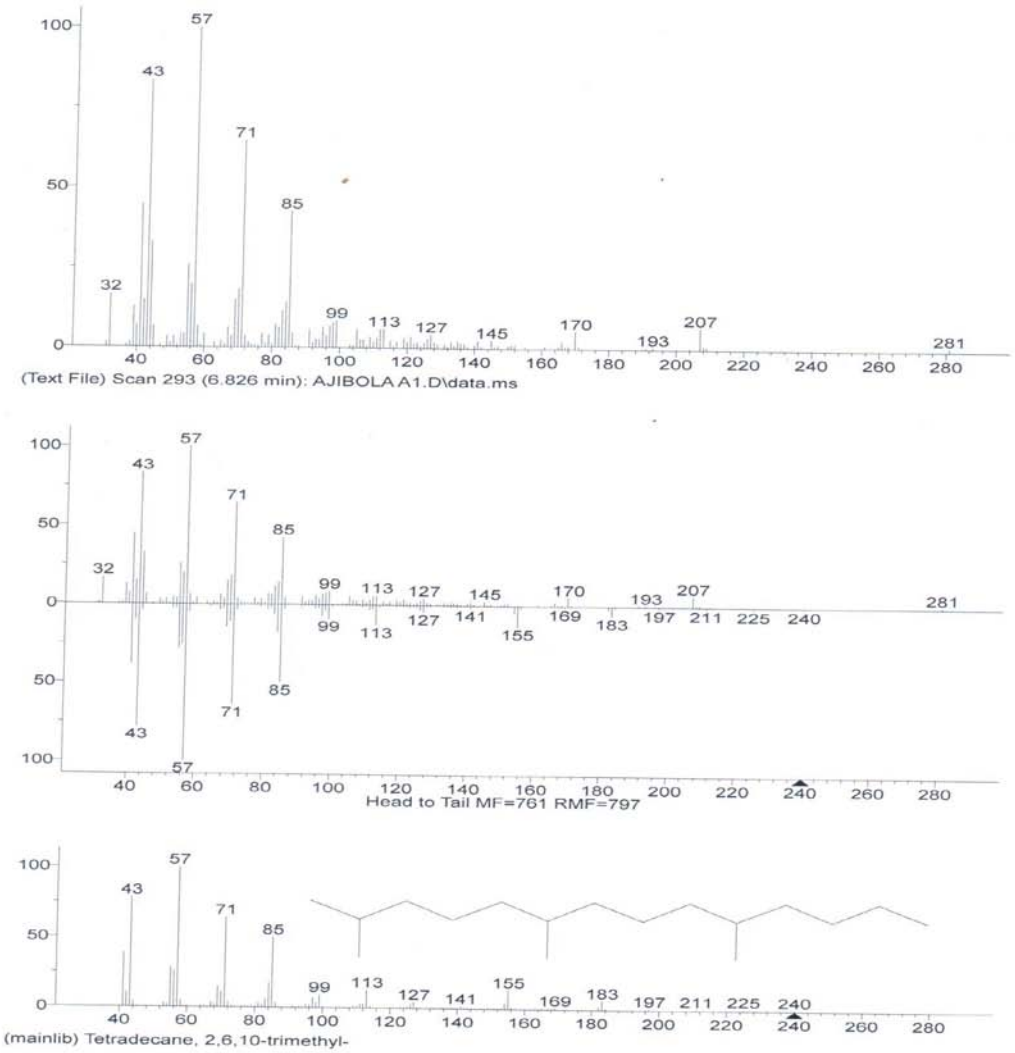

Figure 13 : Showing spectral of compound identified

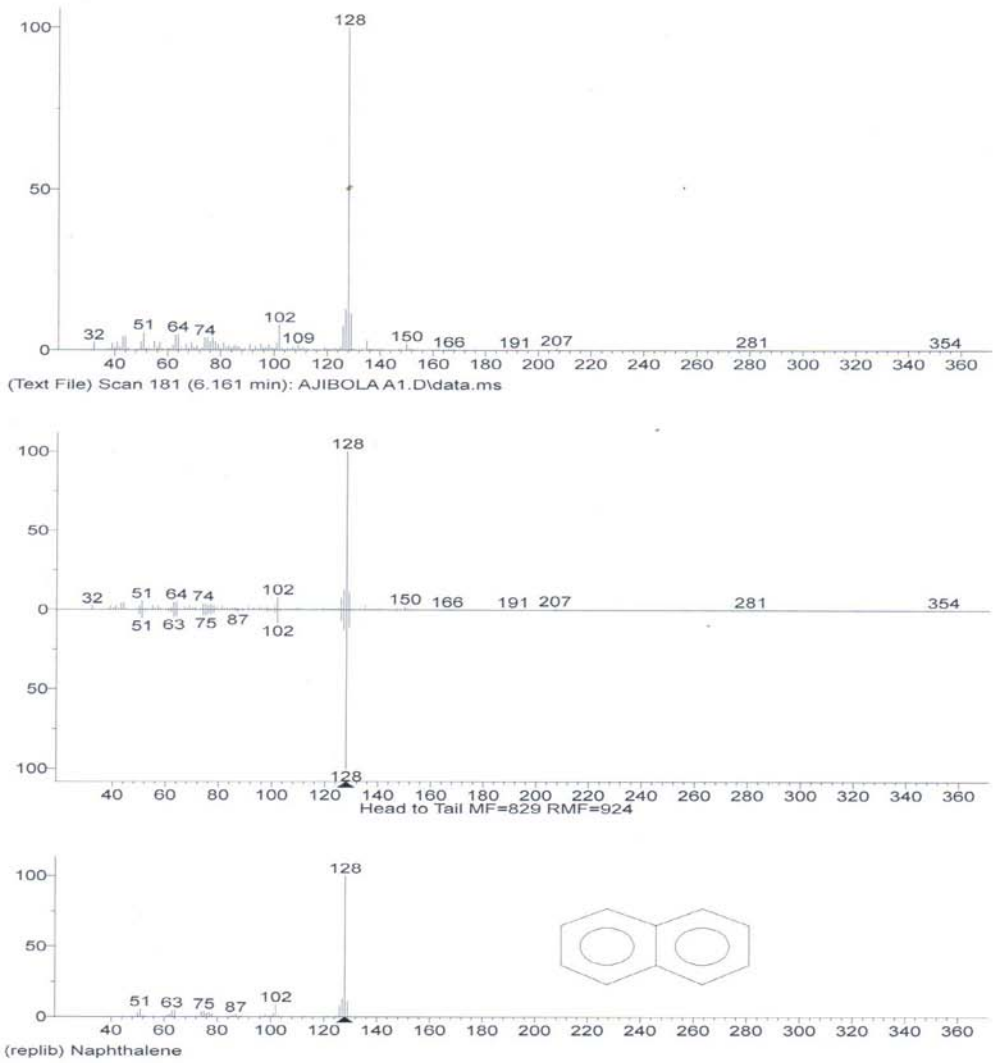

Figure 14 : Showing spectral of compound identified

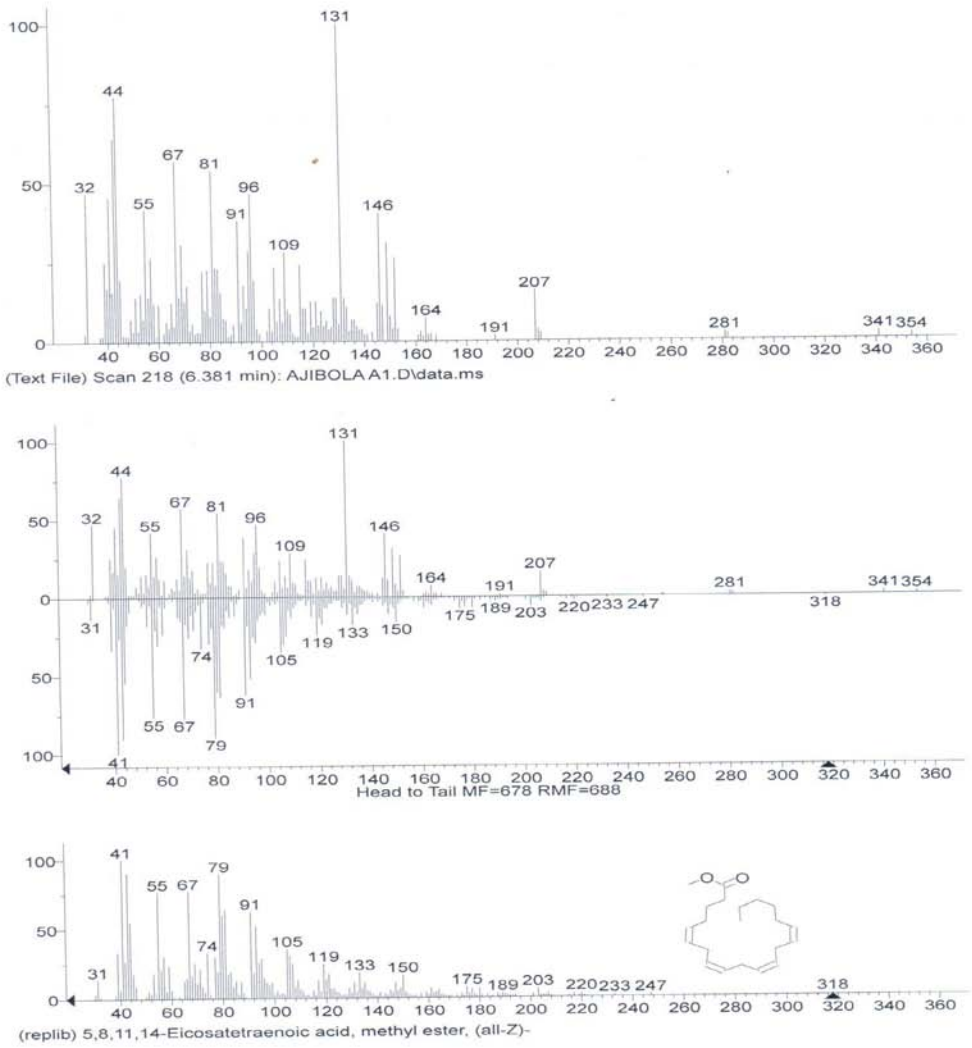

Figure 15 : Showing spectral of compound identified

IV. Discussion

Naphthalene having retention time 6.161 minutes, percentage total 11.369 %, was revealed. Naphthalene has been identified as new range of potent antimicrobials effective against wide range of human pathogens (Rokadeand Sayyed 2009). They occupy a central place among medicinally important compounds due to their diverse and interesting antibiotic properties.(Rokade andsayyed,2009}. Several naphthalene containing drugs are available, such as nafacillin, naftifine, tolnaftate, Also identified in the fraction was P-cymene having retention time of 5.170 minutes, percentage of total 16.439 % was revealed, this compound has just been patented and useful as nutraceutical composition for cognition -cognitive functions and psycho-social status, such as learning, memory and alertness, psychotic stability and maintenance (Ann, 2010). Benzene 1, 2, 4, 5-tetramethyl having retention time 5.117 minutes and percentage of total 6.505 % was identified. Many compounds that have benzene ring have been synthesized and possess strong pharmaceutical activities such as Aspirin, sulfanilamide, amphetamine, acetaminophen. 2-isopropylidene-5-methylhex-4-enal having retention time 5.508 minutes and percentage of total 5.446 %. From the figure 3 showing the chromatogram of the fraction, it is found that p-cymene having % of total 16.439% was the highest followed by Naphthalene of % total 11.369 %, n- Nonadecanol-1 of

% total 10.302 %. Understanding the biochemistry of alcohol metabolism has helped to develop treatments for alcohol abuse. For example, the drug Antabuse inhibits aldehyde dehydrogenase allowing a toxic accumulation of acetaldehyde to occur when alcohol is consumed(Brick, 2003; Brick and Erickson, 1999; Crews,2003; Pohorecky, L. and Brick).Trans-Decalin, 2-methyl of % total 9.468 %. These compounds have been found to have various pharmaceutical applications, this include as anagelsic, antimicrobial activities and as composition in the formulation of drugs and other industrial compounds.

V. Conclusion

From the above analysis, the fungus, P. Notatum has been found to be a reservoir of many bioactive compounds. if these compounds could be isolated and characterized using various spectroscopic techniques, novel and lead candidates compounds may be discovered.

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